Emerging jasmonate transporters

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Abstract: Jasmonic acid (JA) and methyl jasmonate (MeJA) are highly mobile and, when applied to intact plants, these compounds powerfully modulate gene expression. Moreover, activation of jasmonate-responsive genes usually occurs in tissues distal to the treatments. It therefore comes as no surprise that there are jasmonate transporters; these proteins are only now emerging and their discovery is important for a number of reasons. Firstly, biologically active jasmonates such as jasmonoyl-isoleucine (JA-Ile) are potent regulators that are made in small quantities and that need to be delivered to the correct cellular and subcellular sites. Secondly, jasmonates act to redirect resources from growth to defense. For example, when shoots are wounded, jasmonates help to coordinate the appropriate growth of roots so that organ growth rates are balanced even when a distal part of the plant is damaged. Moreover, some plants (Arabidopsis is an example) produce large quantities of the JA-Ile precursor JA upon wounding. JA and/or its immediate precursor can be transported from cell to cell and even from shoot to root (Gasperini et al., 2015; Figure 1A–1C). The mechanisms underlying this transport need to be identified. However, a difficulty for the jasmonate field is that many studies of JA/JA-Ile transport have used exogenous jasmonates applied at sometimes non-physiological levels to plant tissues. These studies can be difficult to interpret, so the discovery of jasmonate transporter mutants represents a welcome breakthrough. Among these is the newly reported jasmonate transporter (JAT1) from Arabidopsis (Li et al., 2017).

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Jasmonic acid (JA) and methyl jasmonate (MeJA) are highly mobile and, when applied to intact plants, these compounds powerfully modulate gene expression. Moreover, activation of jasmonate-responsive genes usually occurs in tissues distal to the treatments. It therefore comes as no surprise that there are jasmonate transporters; these proteins are only now emerging and their discovery is important for a number of reasons. Firstly, biologically active jasmonates such as jasmonoyl-isoleucine (JA-Ile) are potent regulators that are made in small quantities and that need to be delivered to the correct cellular and subcellular sites. Secondly, jasmonates act to redirect resources from growth to defense. For example, when shoots are wounded, jasmonates help to coordinate the appropriate growth of roots so that organ growth rates are balanced even when a distal part of the plant is damaged. Moreover, some plants (*Arabidopsis* is an example) produce large quantities of the JA-Ile precursor JA upon wounding. JA and/or its immediate precursor can be transported from cell to cell and even from shoot to root (Gasperini et al., 2015; Figure 1A–1C). The mechanisms underlying this transport need to be identified. However, a difficulty for the jasmonate field is that many studies of JA/JA-Ile transport have used exogenous jasmonates applied at sometimes non-physiological levels to plant tissues. These studies can be difficult to interpret, so the discovery of jasmonate transporter mutants represents a welcome breakthrough. Among these is the newly reported jasmonate transporter (JAT1) from *Arabidopsis* (Li et al., 2017).

The strategy that Li et al. (2017) used to identify JAT1 was based on the fact that members of the ABC transporter superfamily are already known to transport auxin, cytokinin, strigolactone, and abscisic acid (ABA) (Hwang et al., 2016). Li et al. (2017) looked for candidate jasmonate transporting ABC proteins in a list of genes that were induced by JA treatment in the stamens of *Arabidopsis*. Five putative ABC transporters were found in this list. Rather than immediately characterizing the effects of the *Arabidopsis* insertion mutants for these genes, Li et al. (2017) exploited a yeast assay to test whether any of the gene products were capable of transporting JA. The authors found that exogenous JA (6 mM) was inhibitory to yeast growth; so, to attempt to reverse this inhibition, they expressed the ABC genes in the yeast cells. The result showed that one of the genes, *AtABCG16*, partially rescued yeast growth in the presence of JA. In addition, yeast cells pre-loaded with tritiated JA (^{3}H-JA) exported this molecule efficiently if they expressed this gene. Next, *Arabidopsis* mutants were used to characterize the physiological role of AtABCG16. The effects of a strong allele (*abcg16-1*) included (a) an increase in plant size relative to the wild-type (WT); (b) reduced sensitivity of root growth to exogenous JA and JA-Ile; (c) decreased expression of jasmonate marker genes (e.g., *JAZ* genes) in response to treatment with exogenous MeJA; (d) enhanced susceptibility to infection with *Pseudomonas syringae*; (e) enhanced susceptibility to the fungus *Botrytis cinerea*; and (f) persistence of a JAZ1-GFP reporter that is quickly degraded when expressed in WT roots treated with exogenous JA. Perhaps the most interesting biological impact of the *abcg16-1* allele was revealed when its male fertility (which is jasmonate-dependent in *Arabidopsis*) was examined. The homozygous *abcg16-1* mutant was fertile; however, when *abcg16-1* was crossed with *jasmonate-resistant1* (*jar1*) mutants that cannot make WT levels of JA-Ile, the resultant +/*abcg16-1*; +/*jar1* hemizygous plants were male-sterile, as was the homozygous double mutant. Given its discovery from a stamen transcriptome study and its effects (in the presence of the *jar1* mutant), it seems likely that *abcg16-1* will prove highly interesting to those studying jasmonate transport in flowers.

Moving to the cellular level, when transiently expressed in *Nicotiana benthamiana*, AtABCG16 was previously found to be localized in the plasma membrane (Yadav et al., 2014). Li et al. (2017) found that AtABCG16/JAT1 localized to both plasma membrane and nuclei. This is surprising, since all ABCGs characterized so far localized to the plasma membrane, where they may exhibit a polar localization (Hwang et al., 2016). To investigate the function of AtABCG16/JAT1, the authors performed transport experiments. Plasma membrane AtABCG16/JAT1 functions were primarily revealed by using assays for ^{3}H-JA accumulation in suspension culture cells. Relative to the WT, the *abcg16-1*-derived cells retained high levels of ^{3}H-JA, and inhibitors of ABC transporters reduced the export of ^{3}H-JA into the growth medium. The authors then used other isotope-based assays to explore the functions of nuclear localized AtABCG16/JAT1. These technically demanding assays gave clear results: nuclei isolated from the WT accumulated significantly more tritiated jasmonoyl-isoleucine (^{3}H-JA-Ile) than did nuclei from *abcg16-1* plants. K_m values were determined for the two transport processes catalyzed by AtABCG16/JAT1: the K_m for ^{3}H-JA-Ile uptake by isolated nuclei was approximately 170 nM, whereas the K_m for ^{3}H-JA export from suspension cells was close to 350 nM. These values are within the same order of magnitude, although JA levels often greatly exceed those of JA-Ile in *Arabidopsis*. The finding that the same transporter can export a hormone precursor (JA) to the apoplast and can also transport the active hormone (JA-Ile) from the cytosol into the nucleus is remarkable and possibly unprecedented.

In summary, AtABCG16/JAT1 acts as a dual exporter: it not only exports JA from the cytosol to the apoplast but also acts as an internal exporter by exporting JA-Ile from the cytosol to the nucleus (Figure 1D). However, it cannot be excluded that the difference between JA and JA-Ile specificity observed in the plasma membrane and nucleus is due to a difference in the transport assays of the two compounds. While for the nucleus direct transport experiments could be performed, loading experiments were undertaken for the plasma membrane. Since JA-Ile is much more hydrophobic than JA, loading is much slower than that for JA. Consequently a much lower amount of JA-Ile than JA was present in the cytosol. This might have given the impression that JA export at the plasma membrane was faster than that of

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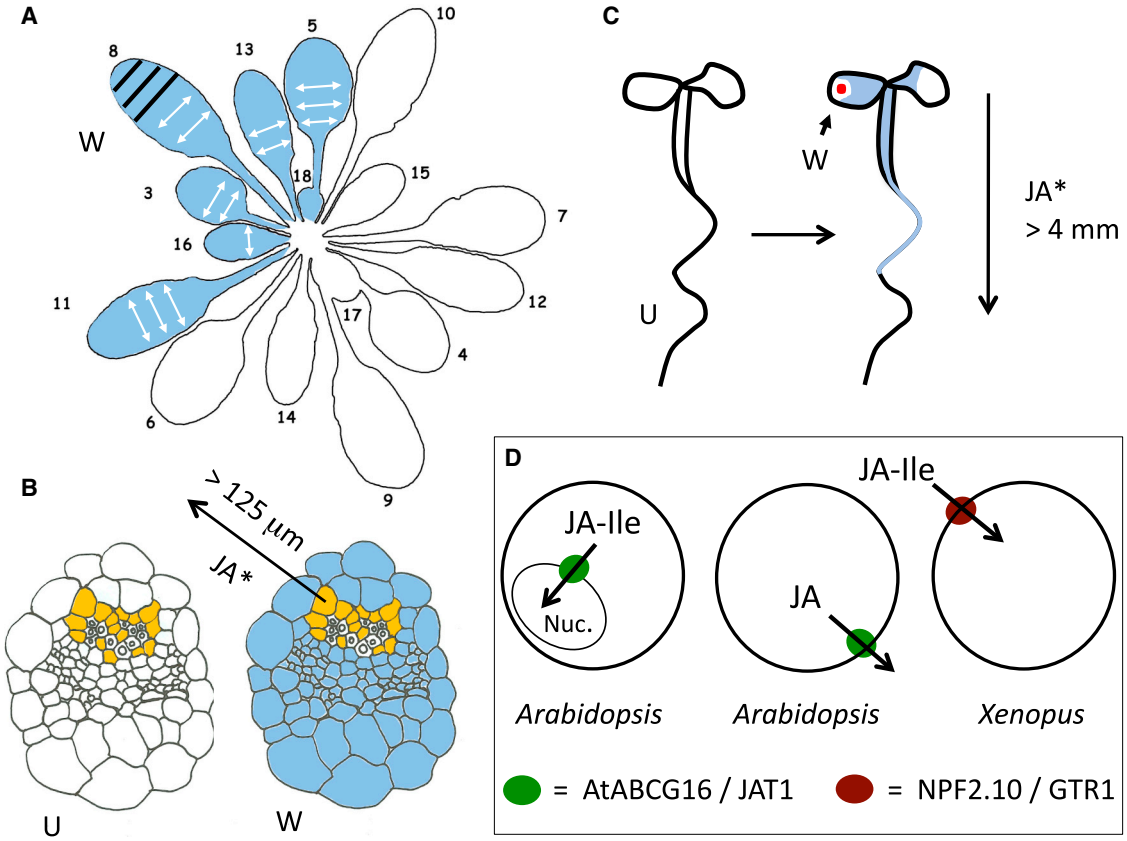

Figure 1. Jasmonate Translocation and Putative Translocators in *Arabidopsis*.

(A) Wounding individual leaves triggers JA accumulation (blue) in distal leaves that share vascular connections with the wounded leaf. JA (and/or JA precursors) made in the vasculature is exported radially in the leaf (white arrows). Leaves are numbered from oldest to youngest; leaf eight was wounded (W).

(B) Small populations of xylem contact cells (orange) produce and export JA (and/or its precursors; blue) radially to distances of at least 125 µm and through at least eight leaf cell layers. Only the vascular cylinder is shown in representations of transversal sections from petioles from unwounded (U) or wounded (W) rosettes.

(C) Seedling grafting experiments have shown that wounding cotyledons or young leaves triggers the synthesis of JA (indicated in blue) in aerial tissues followed by the axial transport of JA and/or its immediate precursor OPC4 (3-oxo-2-(2-(*Z*)-pentenyl)-cyclopentane-1-butanoic acid) through the hypocotyl to the root over distances of at least 4 mm. The red dot on a cotyledon indicates a wound. U, unwounded; W, wounded. JA* in **(B)** and **(C)** indicate that JA and/or JA precursors are mobile. **(A)**, **(B)**, and **(C)** summarized from Gasperini et al. (2015).

(D) Putative roles of AtABCG16/JAT1 and NPF2.10/GTR1. AtABCG16/JAT1 transport functions were characterized in *Arabidopsis* (Li et al., 2017), whereas NPF2.10/GTR1 transport functions were characterized in *Xenopus* oocytes (Saito et al., 2015). The two transport functions of AtABCG16/JAT1 are represented in different cells; it is not yet known whether they both operate within the same cell type. NPF2.10/GTR1 located to the plasma membrane when expressed in onion epidermal cells (Nour-Eldin et al., 2012).

JA-Ile. Alternatively, a nuclear or plasma membrane protein may interact with the transporter and modulate transport activity. Transport experiments with the corresponding membrane vesicles could resolve this question.

AtABCG16 belongs to a clade of ABCG half-size transporters that has not been described with the exception of one report on AtABCG16 (Ji et al., 2014). Using genome-wide association mapping, these authors identified *AtABCG16* as a candidate gene important to confer resistance to *P. syringae*. However, due to the ABA-inducible expression of *AtABCG16* and the reduced tolerance to exogenous ABA seen in *abcg16* insertion mutants, the authors suggested that AtABCG16 could be involved in ABA transport or signaling. Since many ABC transporters exhibit a broad range of substrates, their interpretation may still be valid. In the work by Li et al. (2017) the most original finding is the JA export function of AtABCG16/JAT1. This is the first such activity described, and this activity of AtABCG16/JAT1 is likely to provide entirely new insights into jasmonate signaling.

How can we put the new results of Li et al. (2017) into the context of what is known about jasmonate transport in *Arabidopsis*? Concerning JA-Ile, a protein capable of importing this molecule (and also gibberellin [GA]) into *Xenopus* oocytes has been discovered previously (Saito et al., 2015). This protein, NPF2.10/GTR1, was identified as a glucosinolate transporter (Nour-Eldin et al., 2012). More recently, Saito et al. (2015) showed that *gtr1* mutants were hypersensitive to MeJA and had shorter stamen filaments and reduced fertility compared with the WT. These later effects of the mutant could be rescued with exogenous GA. A recent follow-up study by Ishimaru et al. (2017) showed that *gtr1-1 null* mutants did not strongly affect wound-induced JA and

JA-Ile levels and did not strongly perturb the expression of jasmonate-regulated genes such as *JAZ1* and *VSP2*. However, the *gtr1-1* mutant reduced the capacity of plants to transport exogenous isotope-labeled JA and JA-Ile from wounded leaves to distal leaves. Therefore, it is possible that there are multiple JA-Ile transporters in *Arabidopsis*. Other possible candidate JA-Ile transporters are NPF8.1/PTR1, NPF8.2/PTR5 and NPF4.1/AIT3 which acted as JA-Ile importers in yeast (Chiba et al., 2015). It will be informative to place the new AtABCG16/JAT1 results in the broader context of jasmonate transport shown in Figure 1 as well as to deepen our knowledge of the molecular roles of this protein in flower development and fertility. To do this, powerful approaches can be based on combining the use of *jat1/jar1* double hemizygous plants with modulated endogenous jasmonate production.

While candidate jasmonate transporters emerge, at least two further proteins might be involved in the transport of the JA precursor 12-oxo-phytodienoic acid (OPDA) in *Arabidopsis*. Firstly, *Arabidopsis* ABC transporter Comatose (AtABCD1/CTS) may import OPDA into the peroxisome as part of its function as a broad specificity peroxisomal fatty acid acyl-coenzyme A (CoA) transporter (Theodoulou et al., 2005; Nyathi et al., 2010). A second candidate for an OPDA transporter is acyl-CoA binding protein 6 (ACBP6), which is encoded by a wound-inducible gene expressed in the phloem. An insertion mutant of *ACBP6* showed greatly increased level of OPDA in phloem exudates from *Arabidopsis* (Ye et al., 2016). ACBP6 is an interesting candidate, potentially affecting OPDA transport or synthesis in phloem, and validation studies are eagerly awaited. The same can be said for the jasmonate transport roles of AtABCG16/JAT1. If confirmed in further work, this protein will be a particularly valuable tool with which to deepen our knowledge of jasmonate biology during floral organ development and in the defense responses of plants.

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